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Within- and among-population genetic variation in the Southern Upland Designatable Unit of Maritime Atlantic Salmon (*Salmo salar* L.)

Variation génétique au sein des populations et entre les populations dans l'unité désignable du saumon de l'Atlantique des hautes terres du Sud, région des Maritimes (*Salmo salar* L.)*

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ABSTRACT

Genetic variation was surveyed at 17 microsatellite loci from 1,039 individuals from 11 rivers of the Southern Upland of Nova Scotia, and several small and large reference populations from elsewhere in Atlantic Canada. Levels of within-population genetic variation were lower in sample collections obtained from rivers of the Southern Upland compared to those obtained from New Brunswick, but generally speaking, similar to or slightly greater than those from endangered populations of the inner Bay of Fundy. Within the Southern Upland, south and southwest sample collections, including Salmon River Digby, Tusket, and Round Hill, were more genetically depauperate, with the latter exhibiting nearly half the level of allele richness observed in the more variable northeast Southern Upland collections. Based on several indicators, contemporaneous sample collections from Medway, St. Mary's East Branch, and Salmon River Guysborough are the most genetically variable of those analyzed here from this Designatable Unit.

Levels of genetic structuring among sample collections from different river populations (excluding within-river sample collections) of the Southern Upland varied from F_{ST} = 0.014 to 0.168, and averaged F_{ST} = 0.054. However, after removal of comparisons involving the highly divergent Round Hill sample collection, average F_{ST} estimates were 0.039, comparable to that reported in other studies over similar geographic ranges. Generally speaking, salmon from the 11 Southern Upland populations clustered into two major sub-groupings, reflecting Salmon Fishing Area (SFA) 21 (Salmon River Digby, Tusket, Medway, LaHave and Gold rivers) and SFA 20 (Musquodoboit, Moser, St. Mary's, Salmon River Digby, and Country Harbour rivers). In the first sub-group, the clustering of sample collections in both phylogenetic and factorial correspondence analyses generally appears to mirror coastal distance, and likely reflects the effects of decreasing effective migration and gene flow with small increases in geographic distance. In the second sub-group, patterns are more complex, with sample collections from neighbouring rivers sometimes exhibiting high levels of differentiation. Sample collections from Musquodoboit and Moser rivers, in particular, are relatively divergent from other sample collections from this sub-group, including each other. Based on levels and patterns of within- and among-population genetic variation only, Medway and either St. Mary's East Branch or Salmon River Guysborough may be important populations for prioritization of conservation efforts, followed by Moser and Musquodoboit river populations.

RÉSUMÉ

La variation génétique a été examinée sur 17 loci microsatellitaires de 1 039 individus de 11 rivières du bas-plateau sud de la Nouvelle-Écosse et de plusieurs grandes et petites populations de référence venant d'ailleurs au Canada atlantique. Les niveaux de variation génétique au sein d'une population étaient plus faibles dans les échantillons recueillis des rivières du bas-plateau sud que dans ceux recueillis au Nouveau-Brunswick, mais globalement ils étaient semblables à ceux de populations en voie de disparition à l'intérieur de la baie de Fundy ou légèrement plus élevés que ceux-ci. Au sein du bas-plateau sud, les échantillons recueillis dans le sud et le sud-ouest, notamment dans les rivières Salmon (comté de Digby), Tusket et Round Hill étaient génétiquement plus défectueux, le dernier endroit ne présentant que près de la moitié du nombre d'allèles observés dans les échantillons plus variables de la partie nord-est du bas-plateau sud. Selon plusieurs indicateurs, les échantillons recueillis en même temps dans la rivière Medway, le bras est de la rivière St. Mary's et la rivière Salmon (comté de Guysborough) sont les plus génétiquement variables de tous ceux analysés de l'unité désignable.

Les niveaux de structuration génétique des échantillons recueillis au sein de différentes populations de rivière (à l'exclusion des échantillons au sein de la rivière) du bas-plateau sud variaient d'un F_{ST} allant de 0,014 à 0,168, et le F_{ST} moyen était de 0,054. Cependant, après l'exclusion des comparaisons comprenant les échantillons très divergents de Round Hill, le F_{ST} moyen estimé était de 0,039, taux comparable à ceux d'autres études couvrant des étendues géographiques semblables. De façon générale, le saumon des 11 populations du bas-plateau sud regroupé dans deux grands sous-groupes, représentant la zone de pêche au saumon 21 (rivières Salmon [comté de Digby], Tusket, Medway, LaHave et Gold) et la zone de pêche du pétoncle 20 (rivières Musquodoboit, Moser, St. Mary's, Salmon [comté de Digby] et Country Harbour). Dans le premier sous-groupe, le regroupement des échantillons recueillis pour les analyses phylogéniques et factorielles de correspondance semble généralement être fonction de la distance à la côte et reflète probablement les effets d'une migration et d'un flux génétique en décroissance avec de petites augmentations dans la distance géographique. Dans le deuxième sous-groupe, les tendances sont plus complexes, avec des échantillons de rivières avoisinantes présentant parfois des niveaux de différenciation élevés. Les échantillons des rivières Musquodoboit et Moser, en particulier, sont relativement différents l'un de l'autre et par rapport aux autres échantillons de ce sous-groupe. Selon les niveaux et les tendances en matière de variation génétique au sein des populations et entre les populations uniquement, les populations des rivières Medway et du bras est de la rivière St. Mary's ou de la rivière Salmon (comté de Guysborough) pourraient devenir prioritaires en ce qui concerne les efforts de conservation, suivies des populations des rivières Moser et Musquodoboit.

INTRODUCTION

Like salmon from elsewhere in the Maritimes, those of the Southern Upland (SU) of Nova Scotia have been impacted by destruction or loss of freshwater habitat, barriers to up- and down-stream fish passage, and, more recently, low marine survival (DFO, 2000). Salmon in many rivers from this region, however, have also long been affected by acid deposition and low pH. Records show declining pH trends for several SU rivers as early as the 1950s (Watt, 1987) and acidification is thought to have resulted in a 50% decline, from 45,000 to 22,700 adult salmon by 1986 (DFO, 2000), with some river populations being more heavily impacted than others. The severity and, importantly, the duration of demographic declines may have led to mild to severe genetic bottlenecks, and population persistence may now be impacted by both demographic and genetic effects of small population size. Possible genetic implications of recent reductions in population size include a) accumulation of inbreeding and immediate loss of fitness due to the expression of recessive deleterious alleles (Charlesworth and Charlesworth, 1987), b) random changes in allele frequency distributions from locally adapted fitness optima (Doyle *et al.*, 2001), and c) reduced heterozygosity and number of alleles, negatively impacting short-term (Nevo, 1978) and long-term (Moritz, 1999) adaptability, respectively.

Examination of patterns of molecular genetic variation can be useful in helping to identify and prioritize remaining within-species biodiversity for conservation actions. For example, analyses of mitochondrial DNA, can help identify major ancestral lineages not otherwise apparent (Utter *et al.*, 1993; Verspoor *et al.*, 2002). Additionally, analyses of patterns and extent of genetic structuring among samples from different locations can provide information on amounts of recent and ongoing gene flow. This information is important in inferring the potential for adaptive differences to have developed between salmon from different rivers or regions, since genetically based adaptive differentiation can only accrue in the absence of large amounts of gene flow (Waples, 1991). Assessments of levels of within-population genetic variation have also been used to prioritize populations for conservation efforts (Petit *et al.*, 1998) with, all else being equal, more weight given to populations exhibiting higher levels of genetic variation. This increased importance of more genetically diverse populations reflects both a) potentially increased likelihood of persistence of a given population over more genetically depauperate populations (Saccheri *et al.*, 1998) and, hence, the ability of a population to contribute demographically to the species through time, and b) the potential contribution to the adaptability of the species in the face of future environmental change.

Here, the results of analyses of genetic variation at a large number of nuclear microsatellite loci (17) from 13 sample collections (representing 11 rivers) from the SU Designatable Unit (DU) of Atlantic salmon recently identified as endangered (COSEWIC, 2010) are presented. The objectives of these analyses are (1) to report and interpret levels of within-population genetic variation (and other measures of genetic health), (2) to quantify the extent and patterns of present-day genetic structuring within the SU DU, and (3) to prioritize populations for conservation measures based on 1 and 2 above.

METHODS

SAMPLE COLLECTIONS

The SU rivers surveyed here include representatives from throughout much of the DU, beginning with Round Hill (ROH00), a southwest river at the extreme periphery of the SU nearest the inner Bay of Fundy (iBoF) assemblage of rivers, continuing counterclockwise around mainland Nova Scotia to include two rivers from the southern shore (Salmon River, Digby (SAD00) and Tusket (TSK99)), and eight rivers along the eastern shore, ending with the Salmon River, Guysborough (SAG09), which is located very near the northeast boundary of the SU DU (Figure 1, Table 1). Three sample collections were obtained from the St. Mary's River, one from throughout the river collected in 2000 (SMA00), a second from the East Branch of the St. Mary's River (smE07) obtained in 2007, and a third from the West Branch of the St. Mary's River (smW07) obtained that same year (Table 1). Samples from nearly all of the other SU rivers were collected in similar years, 1999-2002, except the SAG09, which was obtained in 2009 (Table 1). All SU sample collections were comprised of parr obtained by electrofishing from multiple dispersed sites. Genetic data from the same 17 microsatellite loci were also included from two large reference populations, the Nashwaak River (NSH00) from the outer Bay of Fundy and the Kedgwick River (RKR03) from the Gulf region, as well as sample collections from two small endangered populations from the Stewiacke (STW01) and Gaspereau (GAK02) rivers of the iBoF.

LABORATORY ANALYSES

Fin clips were collected and stored in 1.5 ml microcentrifuge tubes containing 1 ml of ethanol. DNA was extracted and purified using Qiagen's 96-well DNeasy kits, following the manufacturer's specifications. Polymerase Chain Reaction (PCR) amplifications were carried out for each locus separately, in 10 µl volumes, containing between 1-100 nanograms of template DNA, 0.2 mM each dNTP, 0.1 µM labelled and unlabelled primers, 1X KCl buffer (10mM Tris HCl, 50 mM KCl, 0.08% Nonidet P40), 0.5 units of Taq DNA polymerase supplied by MBI Fermentas and 2.5 mM MgCl2. Primer sequences for loci *Ssa*85, *Ssa*171, *Ssa*197 and *Ssa*202 are given in O'Reilly *et al.* (1996); SS*sp*2201, SS*sp*2210, SS*sp*2215, SS*sp*2216, SS*sp*1G7 and SS*sp*1605 are given in Paterson *et al.* (2004); *Ssa*D58, *Ssa*D144, *Ssa*D71, and *Ssa*D486 in King *et al.* (2005); MST 3 in Presa and Guyomard (1996); and *Ssos*L417 in Slettan *et al.* (1995). Primers for the locus *Ssa*D85 are unpublished, but are CTTTGGCTGTTTCAGGTATGAC and CACTGCTCTACAACAGAAGTCTC (T. King, Genbank Accession AF525213). Thermal cycling conditions were as follows: (94°C for 3 min)X1, (94°C for 45 sec, 58°C for 30 sec, 74°C for 1 min)X9, and (94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min)X 27, followed by a 30-min extension step at 72°C. PCR amplification products from individual loci were size-fractionated without purification, or products from multiple loci were combined in various groups, and salt, unincorporated dNTP's and non-labeled primers removed using either Qiagen's 96-well MinElute plates (as specified by the manufacturer) or using ethanol precipitation methods, prior to size fractionation. Alleles were size-fractionated using denaturing gradient electrophoresis, and detected using either an MJ Research Base station or an Applied Biosystems 3130XL. Size determinations of fragments analyzed in different batches, on different days and on different platforms, were cross-standardized by including two of ten individuals with known genotypes in each batch of samples analyzed; these two individuals varied across batches, providing an "internal label", in addition to an external written label as normally used, to identify each set of samples analyzed. Ten samples from each batch of 84 were analyzed twice to identify potential sample placement errors, strip inversions, plate inversions and other laboratory mistakes.

ESTIMATION OF WITHIN-POPULATION GENETIC VARIATION

Several different measures of within-population genetic variation were estimated for each sample collection and each locus, and values averaged across all 17 loci. The *observed number of alleles, Obs#A,* was simply the number of different alleles observed in a given sample collection, with no attempts to control for sample-size effects. To permit comparisons of numbers of alleles observed across sample collections of varying size, the standardized number of alleles or *allele richness* (*AR*) was estimated using *FSTAT* version 2.9.3.2 (Goudet, 1995, 2001), which is based on the rarefaction procedure of Hurlbert (1971). In this approach, estimates of the expected number of different alleles for each population are made by repeated sampling of 2N genes, where N is the smallest sample size of diploid genotypes present among the populations under study (26 in this study). The *observed heterozygosity, Ho*, was simply the proportion of genotypes exhibiting two different alleles. *Gene diversity, GD*, was also estimated using *FSTAT*, and is the likelihood that two alleles randomly drawn from a sample are different. The extent of non-random mating, F_{IS}, (*f* from Weir and Cockerham, 1984), approximately equal to (Hs-Ho/Hs), where Hs = GD, and significance of departures from zero were estimated using Genetix version 4.02 (Belkhir *et al.*, 2001).

ANALYSIS OF LEVELS AND PATTERNS OF AMONG-POPULATION GENETIC VARIATION

Weir and Cockerham's (1984) F_{ST}, and significance of deviations from zero (homogeneity), were estimated for all pairs of sample collections using the program Genetix version 4.02. The program Populations 1.2.28 (Langella, 2005) was used to estimate Nei's pairwise D_A distances, to construct neighbour-joining unrooted phylograms of D_A distances between sample collections. Levels of confidence of phylogenetic groupings were estimated using bootstrapping methods implemented in the program Populations 1.2.28, resampling across loci 1,000 times. Output was visualized using the program TreeView 1.6.6 (Roderic, 2001). Factorial Correspondence Analysis (FCA), adapted for use for molecular genetic data (She *et al.*, 1987) and implemented here using the program Genetix version 4.02, was carried out to visualize the relative similarity of genotypes of individual salmon from different populations. FCA is particularly useful here because it makes no *a priori* assumptions about population membership, providing (a) insight into possible unknown sub-structuring within previously assumed populations, and (b) resolution of similarity between subsets of individuals in different pre-defined populations, such as strays or recent immigrants from location B now residing in location A, and native salmon in location B. A hierarchical analysis of molecular genetic variation (AMOVA) was carried out using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010) to estimate the amount of genetic variation partitioned within versus among sample collections.

RESULTS AND DISCUSSION

WITHIN-POPULATION GENETIC VARIATION IN SOUTHERN UPLAND ATLANTIC SALMON

Overall, sample collections obtained from the SU are much less genetically variable than those from the large reference populations analyzed here. Fewer *observed numbers of alleles* (averaged across loci surveyed) were found in nearly all SU sample collections compared to NSH00 and RKR03, despite the fact that this metric is sensitive to sample size, and many SU sample collections were larger than those of the NSH00 and RKR03, particularly the latter (Tables 1 and 2). Mean *allele richness*, a measure of the number of alleles in a given sample collection (averaged across loci) that controls for the effects of variable sample size, was also lower in all SU sample collections compared to NSH00 and RKR03, markedly so in some instances. Mean *gene diversity*, approximately equivalent to the likelihood that two alleles drawn

at random from a population at a given locus are different (averaged across loci) were also lower in all SU sample collections compared to NSH00 and RKR03 population samples, as was, in most cases, mean *Observed heterozygosity*, the proportion of individuals exhibiting two different alleles at a given locus, averaged across loci. The above differences in *allele richness, gene diversity*, and *observed heterozygosity* between this group of large reference populations and the group of SU samples were all significant (p = 0.008, 0.005, and 0.039, respectively; FSTAT, one-tailed tests). Average levels of *allele richness*, *gene diversity* and *observed heterozygosity* were, however, higher in most SU sample collections compared to the two small iBoF reference sample collections analyzed here, though differences between these two groups were not statistically significant (p = 0.1370, 0.1840, and 0.1960, respectively; FSTAT, one-tailed tests).

Within the SU, levels of genetic diversity varied greatly, with average *gene diversity* ranging from 0.676 in ROH00 to 0.845 in COU00, average *observed heterozygosity* from 0.725 in ROH00 to 0.859 in MSQ00, and average *allele richness* from 7.39 in ROH00 to 13.32 in SAG09 (Table 2). Not only was ROH00 the most genetically depauperate sample collection based on all three indices, but reductions relative to all other populations were often very large. Average *allele richness* in this population was a little over half (53.4%) of that observed in sample collections from the large reference populations (NSH00 and RKR03), approximately 56% of more variable SU sample collections (MED01, SMA00, smE07, and SAG09), and 64% of the relatively depauperate set of SU sample collections, SAD00 and TSK99 (discussed below). *Gene diversity*, a measure much less sensitive to effects of population bottlenecks, was approximately 22.5%, 18.6%, and 17.5% lower in ROH00, respectively, than averages for sample collections from the large reference populations, the group of more variable SU sample collections, and the set of sample collections from the less variable SU populations. Interestingly, average *observed heterozygosity*, though low compared to other sample collections, was higher than *gene diversity* (also known as expected heterozygosity) in this sample, and nearly all single-locus F_{IS} values (a measure of the extent of inbreeding due to non-random matings) were negative, with multiple F_{IS} values strongly negative, less than -0.075 (Table 2). When effective population size is very small, chance differences in allele frequencies between males and females develop, resulting in an excess of heterozygotes in the offspring. Indeed, Cornuet and Luikart (1996) have developed a method of detecting population bottlenecks based on the existence and extent of heterozygote excess. Given levels of *allele richness* and *gene diversity* relative to other populations, and despite negative F_{IS} values observed, levels of inbreeding due to population bottlenecks and loss of alternate alleles may actually be accumulating in this population and could be higher than in surrounding SU populations. The sample collection from the geographically closest southwest Nova Scotia population, SAD00, was also less genetically variable relative to other samples, exhibiting the second lowest *gene diversity* (0.800) of any population surveyed, and the second lowest *allele richness* of any SU sample (11.51). The TSK99 sample collection, also in southwest Nova Scotia and from the next geographically proximate river, also exhibited similarly low levels of *allele richness* (11.62), the most sensitive indicator of genetic bottlenecks, but surprisingly, levels of *gene diversity* (0.838) and *observed heterozygosity* (0.857) were among the highest for any SU sample collection. The higher level of *observed heterozygosity* relative to *gene diversity* and large number of negative F_{IS} values could again reflect chance differences in allele frequencies between males and females resulting from low effective number of breeders and population bottlenecks (Cornuet and Luikart, 1996; Luikart and Cornuet, 1999).

Several other SU sample collections from outside of southwest Nova Scotia also exhibited lower values of *allele richness*, again the more sensitive indicator of population bottlenecks, though *gene diversity* estimates were relatively high in all these samples. Briefly, *allele richness* estimates were relatively high (above 13.0) in MED01, SMA00, smE07 and SAG09, and

intermediate (11.92-12.61) in LAH00, GLD01, MOS00, smW07 and COU00 collections, with only MSQ00 exhibiting nearly as low levels (11.63) as observed in SAD00, TSK99 and small iBoF reference sample collections. The average *observed heterozygosity* in MSQ00 was higher than any other SU sample collection, and greater than *gene diversity* (also known as expected heterozygosity) for this same population, which is reflected by the many negative single-locus F_{IS} values (Table 2); this is again possibly due to chance differences in allele frequencies between their male and female parents.

Within-population genetic variation in the Salmon Digby, LaHave, Gold, Country Harbour, St. Mary's East Branch, and St. Mary's West Branch rivers in addition to several reference populations, including the Margaree, Philip, and Gaspereau rivers, was also analyzed by McConnell *et al.* (1997). Sample collections were composed of parr obtained a few years prior to 1995. Sample sizes were much smaller than those reported in the present study (20-37, $\bar{x}$ = 31.15, versus 28-84, $\bar{x}$ = 57.7). Additionally, genetic variation was assayed at eight microsatellite loci in their paper versus 17 here, and the overall set of loci analyzed in this study was more variable. Therefore, direct comparisons between populations across years over all loci analysed in these two studies would not be meaningful. However, relative comparisons of levels of genetic variation among populations or groups of populations between the two studies, may be useful. Note that McConnell *et al.*'s estimate of *unbiased heterozygosity*, calculated following Nei (1978), is comparable to *gene diversity* estimated here. Although these authors did not estimate *allele richness*, comparisons of *observed number of alleles* between sample collections or groups of sample collections in their study were equivalent when contrasting samples with identical (or very similar) sample sizes. Fortunately, two microsatellite loci (*Ssa*171 and *Ssa*197) were common to McConnell *et al.* (1997) and this study, thus permitting direct comparisons of gene diversity (but not allele richness) between collection years for populations common to the two studies. Although it is recognized that this is an admittedly small set of loci from which to be drawing inferences, when combined with comparisons of relative levels of genetic variation among populations and between groups of populations between the two studies (both based on a larger number of genetic markers), these analyses collectively may provide additional insight into changes in levels of genetic variation through time.

Gene diversity in the SU sample collections common to both studies (excluding SAD, see below for discussion) were very similar in sample collections from the early 1990s (0.73-0.74) and 2000 (or after) from these same locations analyzed here (0.82-0.83). In other words, the extent of among-population variation in gene diversity in these specific SU populations was minimal, and this has not appeared to have change markedly in the intervening years. In the early 1990s, *gene diversity* in these SU populations was 5% lower than the large Margaree and Philip river collections from the Gulf (0.77), but more variable than the Gaspereau sample also analyzed at that time (0.68). Relative ranking of the large external Gulf, small iBoF, and SU sample collections has not changed in the present study.

In the early 1990s, average *gene diversity* was lower in the Salmon River Digby sample (0.71) relative to the average for the other five SU sample collections common to the two studies (0.732), by approximately 3.0%. Today, genetic variation in SAD00 is 3.5% below the average for these five populations. *Gene diversity* in Salmon River Digby salmon at locus *Ssa*171 has declined from 0.88 to 0.84 and from 0.79 to 0.74 at *Ssa*197 between the early 1990s and 2000, which, averaged across these common loci, represents an approximate 5.5% decline. During this same period, *gene diversity* decreased in four single-locus comparisons among remaining SU sample collections analyzed in the two studies, but increased in five single-locus comparisons, with no change observed in one comparison. Across all five non-SAD populations analyzed by these two studies at these two loci, the decline was 0.003%, which was very minor.

Looking within the St. Mary's River, smW07 exhibited lower levels of *gene diversity*, *observed heterozygosity*, and *allele richness* relative to smE07 and SMA00; all three metrics were very similar between SMA00 and smE07 sample collections. Differences in *gene diversity* and *observed heterozyosity* levels between the two branches of the St. Mary's River were significant (p<0.0224 and p<0.0101, respectively, Wilcoxon signed-rank test, one tailed), and the difference in *allele richness* was almost significant (p<0.07). Interestingly, the St. Mary's East and West branches sample collections analyzed by McConnell *et al.* (1997) exhibited identical levels of *gene diversity* (0.73), and the average number of observed alleles (equivalent to *allele richness* here because the relevant sample sizes were the same in their study) was actually higher in the West Branch of the river (12.38 versus 10.88), though not significantly so (p>0.05, Wilcoxon signed-rank test, one tailed, analyses performed here). There was also an average net loss of genetic variation at *Ssa*171 and *Ssa*197 from the early 1990s to 2007 (0.3%), though this change was small. Taken together, these results indicate that genetic variation had declined on the St. Mary's West Branch (though not the East Branch), and this reduction has occurred over three or four salmon generations. The limited duration over which this change occurred, and the finding of reduced *gene diversity* (relatively insensitive to small to moderate genetic bottlenecks), indicate that the effective population size of this group of individuals was recently quite small, and that salmon in the West Branch may be somewhat reproductively isolated from East Branch salmon of the St. Mary's River.

In summary, salmon from the SU are genetically less variable than salmon from large, nearby, relatively healthy populations from New Brunswick. Although based on very few loci in direct temporal comparisons, and a modest to large number of loci in relative among-group comparisons, the modest reductions observed for the more variable SU populations have occurred prior to the 1990s, and are probably a result of long-term demographic effects of acid precipitation and population reduction. Within the SU, sample collections from southwest Nova Scotia, including TSK99, SAD00 and ROH00 are much less genetically variable than those from rivers along the Eastern shore, and from sample collections from small reference populations from the iBoF. Comparisons with other studies, and analyses presented here, indicate that a portion of the reduced variation reported for at least the SAD population has occurred between the early 1990s and 2000. ROH00 is especially genetically depauperate, and shows several indicators of genetic effects of extreme population bottlenecks. There are also some indications (*allele richness* and heterozygous excess) that MSQ00, and to a lesser extent MOS00, have experienced moderate reductions in genetic variation. MED01, smE07 and SAG09 appear relatively genetically variable, and show fewer indications of recent effects of demographic declines and genetic bottlenecks. However, some evidence exists for reductions in variation in the West Branch of the St. Mary's River, and once again, this loss of genetic variation appears to have occurred quite recently, within three to four salmon generations.

AMONG-POPULATION STRUCTURING IN SOUTHERN UPLAND ATLANTIC SALMON

In a hierarchical analysis of molecular genetic variation (AMOVA), a modest (4.67%) though highly significant (p<0.001) amount of overall genetic variation was partitioned among SU sample collections, with the remaining (95.33%) partitioned among individuals within samples. Pairwise estimates of F_{ST} between sample collections from different rivers ranged from 0.014 to 0.168 ($\bar{x}$ = 0.054) (Table 3) and all pairs of populations were significantly different after corrections for multiple tests (p<0.05). Pairwise estimates involving ROH00 were consistently by far the greatest of those reported here (0.130-0.168, $\bar{x}$ = 0.141), higher than typically observed for this species in North America over much greater geographic distances (McConnell *et al.* 1997; King *et al.*, 2001; Vandersteen Tymchuk *et al.*, 2010). Given the very low levels of genetic variation observed in this population, and indications of possible genetic bottlenecks, this high degree of genetic divergence may reflect rapid recent drift and not the degree and extent of

long-term reproductive isolation. All other among-river comparisons (excluding the smE07 and smW07 comparison, which involves sample collections within a given river) ranged from 0.014 to 0.063 ($\bar{x}$ = 0.039) (Table 3), which are more in line with values reported in other studies of microsatellite variation in North American Atlantic Salmon over a similar geographic scale (McConnell *et al.*, 1997; King *et al.*, 2001; Vandersteen Tymchuk *et al.*, 2010).

Excluding ROH (located in SFA 22), genetic differentiation among sample collections from southwest SU and southern eastern shore SU populations (corresponding roughly to SFA 21) was minimal, generally between 0.025 and 0.035 (Table 3). This genetic similarity (based on F_{ST} values) was also reflected in the phylogram of pairwise estimates of Nei's D_A distances; all five populations in SFA21 group together before clustering with MSQ00 and the remaining populations (Figure 2). In FCA of individual multilocus genotypes, salmon from these five populations also grouped into one of two main clusters of individuals observed in this study (Figure 3); this same pattern was reflected in FCA of sample collections or populations (data not shown). Interestingly, in all these analyses, MED01, geographically situated between SAD00 and TSK99 to the southwest, and LAH00 and GLD01 to the northeast (Figure 1), is genetically intermediate (Table 3, Figures 2 and 3) to these sets of sample collections. Neighbouring TSK99 and SAD00 exhibit minimal genetic differentiation and group out together in multiple analyses, as do geographically neighbouring GLD01 and LAH00 (Figures 1, 2, and 3). With the exception of SAD00, which actually separates somewhat from these remaining four populations along Axis 3 in FCA (not readily visible in this image), salmon from SFA 21 generally cluster together by population, and in a very clear and directional pattern with minimal overlap between geographically neighbouring populations, and almost no overlap of individuals from more geographically distant populations. Specifically, individuals from TSK99 (purple cubes) cluster together at the upper left and back of the graph, with individuals from MED01 (white cubes), then LAH00 (grey cubes) dropping and moving to the right, with GLD01 (pink cubes) far forward and near the bottom, close to the center point (0) of Axis 1 (Figure 3). The slight deviation of SAD00 salmon in individual FCA may again reflect recent drift due to genetic bottlenecks, suggested by analyses of within-population variation discussed above. Overall, this pattern closely parallels the geographic juxtapositioning of these populations based on coastal distances (Figure 1). If these populations are in equilibrium, with patterns of genetic variation reflecting recent and ongoing demographic events rather than historic patterns of colonization, these results likely reflect effective migration and gene flow, which would appear to decrease with increasing geographic distance. However, this observed pattern may also reflect poor survival of offspring of more distant strays in more ecologically divergent environments, although this would presume that relevant environmental variables are similarly diverging with increasing geographic distance. These results suggest that small effective population size and rapid genetic drift have not dramatically impacted allele frequency distributions of these four populations, potentially driving them from local fitness optima.

Interpretation of patterns of among-population genetic structuring in the remaining salmon from the SU, those belonging to SFA 20, is more complex. MOS00 is geographically very close to the SMA00, and more proximate to the remaining northeast SU rivers sampled here, yet is fairly divergent from SMA00; F_{ST} = 0.045 (Table 3). Note, too, that MOS00 is very divergent from SMA00 in phylogenetic analyses based on Nei's D_A distance, and individuals from this population cluster with each other but apart from all other SFA 20 salmon in FCA (Figures 2 and 3, respectively). MOS00 is also highly divergent from its other neighbour, MSQ00 (F_{ST} = 0.058), located to the southwest. On the other hand, pairwise F_{ST} estimates between MSQ00 (located at the southwest extreme of SFA 20) and SMA00 were modest (0.027) (Table 3) and MSQ00 and SMA00 salmon cluster together in FCA (Figure 3). Excluding MSQ00 and MOS00, sample collections from SFA 20 generally exhibited minimal differentiation, modest F_{ST} values and minimum separation in FCA (Table 3, Figure 3).

Within the St. Mary's River, samples obtained from the two main branches (smE07 and smW07) were less divergent than all between population comparisons (F_{ST} = 0.008) though this value was significantly greater than zero at $p<0.05$ after corrections for multiple tests. This, and the observation of statistically significant differences in levels of within-population genetic variation between these two samples reported above, suggest a degree of reproductive isolation between East and West branch salmon. Note that F_{ST} values between either branch of the St. Mary's River collected in 2007 and SMA00 were moderately high, and greater than between-branch differences. Additionally, that smW07 was more divergent from SMA00 than smE07; this result is consistent with above reports of loss of genetic variation in the West Branch, small effective population size, and increased rates of temporal change due to genetic drift.

In summary, excluding ROH00, a modest but significant amount of among-population genetic structuring was observed across the SU Atlantic Salmon analyzed here. Although every sample collection was statistically different from every other, there is some evidence for the increased similarity of southwest and southeastern shore populations, and patterns of gene flow among these populations appear, generally speaking, to mirror geographic proximity (Figures 1, 2, and 3). Sample collections from northeast SU are more differentiated from each other (based on pairwise F_{ST} estimates), though this largely reflects increased differentiation of MSQ and MOS from SU salmon; SMA00, COU00 and SAG09 are much less divergent from each other, and may constitute a second major grouping within the SU. An important caveat in the interpretation of the above results is that a component of the among-population divergence reported could be due to both a) sampling error, expected to contribute noise equivalent to $1/2S$, where S = number of individuals analyzed in a given population (Waples, 1998; Garant *et al.*, 2000), and b) undetected kinship, expected to contribute noise at a level equivalent to $1/2Nb$, where Nb = the effective number of breeders (Allendorf and Phelps, 1981; Waples, 1998; Garant *et al.*, 2000).

IMPLICATIONS OF OBSERVED WITHIN- AND AMONG-POPULATION GENETIC VARIATION FOR THE PRIORITIZATION OF CONSERVATION EFFORTS FOR SOUTHERN UPLAND ATLANTIC SALMON

All salmon populations from a given region potentially contribute genetically or demographically to the long-term persistence of a given DU and possibly the species itself, and are therefore important. However, resources for biological conservation are sometimes scarce, and undesirable decisions, often of great risk, must be made. A number of different approaches have been suggested for prioritizing species for conservation, recently discussed in O'Reilly and Doyle (2007). Ultimately, decisions would ideally be based on many criteria, including a) molecular genetic and genetically based phenotypic differences in quantitative traits (Crandall *et al.*, 2000), and b) ecological and life history information (Utter *et al.*, 1993). Here, only results from analyses of neutral molecular genetic data are presented, recognizing that this is only part of the picture. Petit *et al.* (1998) suggest an approach that prioritizes populations based on within-population genetic variation (specifically, AR) and divergence among populations, and, hence, what each contributes most to the total diversity of a given group of populations. Initial analyses, based on the criteria of Petit *et al.* and molecular genetic data presented here, would suggest the Medway population could be important as it represents the major phylogenetic grouping of southwest Nova Scotia/southern eastern shore populations, and exhibits the highest level of allele richness for this sub-group. For the remaining northwest populations, St. Mary's East Branch and Salmon River Guysborough both exhibit high levels of AR, and either might well represent this sub-group of populations. Moser and Musquodoboit are both relatively divergent from all other populations (and each other) and could be important.

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Table 1. Sample collection information, including river code, collection year, location, assigned Designatable Unit (DU) and sample size.

Sample collection name	Sample collection code	Collection year	Lat/Lon of river mouth	Assigned DU	Sample size
Nashwaak River	NSH00	2000	45-57-20; 66-37-18	oBoF	70
Stewaicke River	STW01	2001	45-19-11; 63-29-03	iBoF	82
Gaspereau River	GAK02	2002	45-07-21; 64;16;34	iBoF	66
Round Hill River	ROH00	2000	44-46-21;65-25-53	SU	28
Salmon River, Digby	SAD00	2000	44-03-09; 66-10-10	SU	44
Tusket River	TSK99	1999	43-50-37; 65-59-05	SU	60
Medway River	MED01	2001	44-08-01; 64-37-34	SU	83
LaHave River	LAH00	2000	44-22-05; 64-29-34	SU	49
Gold River	GLD01	2001	44-32-48; 64-19-02	SU	84
Musquodoboit River	MSQ00	2000	44-47-25; 63-07-42	SU	53
Moser River	MOS00	2000	44-58-16; 62-15-12	SU	58
St. Mary's (main stem)	SMA00	2000	45-08-18; 61;59-12	SU	79
St. Mary's East Branch	smE07	2007	45-15-29; 62-04-09	SU	59
St. Mary's West Branch	smW07	2007	45-15-29; 62-04-09	SU	41
Country Harbour River	COU00	2000	45-14-39; 61-48-13	SU	42
Salmon River, Guysborough	SAG09	2009	45-21-08; 61-30-14	SU	30
Kedgwick River	RKR03	2003	47-39-51; 67-29-29	CU19	58

Notes:
1. oBoF = outer Bay of Fundy; iBoF = inner Bay of Fundy; SU = Southern Upland; ECB = East Cape Breton.
2. CU19 = identified in DFO and MNRF (2008) as CU-19, former SFA 9.
3. Lat/Lon of river mouth = degrees-minutes-seconds N; degrees-minutes-seconds W.

Table 2. Measures of within-population genetic variation and F_{IS} (inbreeding coefficient) estimates for Southern Upland and several other reference sample collections.

	Sample collection																	
	NSH 00	STW 01	GAK 02	ROH 00	SAD 00	TSK 99	MED 01	LAH 00	GLD 01	MSQ 00	MOS 00	SMA 00	smE 07	smW 07	COU 00	SAG 09	GRA 10	RKR 03
N	70	82	66	28	44	60	83	49	84	53	58	79	59	41	42	30	53	58
GD Avg.	0.870	0.808	0.803	0.676	0.800	0.838	0.829	0.822	0.833	0.841	0.813	0.835	0.827	0.819	0.845	0.832	0.852	0.874
GD Var.	0.008	0.015	0.020	0.031	0.038	0.012	0.027	0.018	0.018	0.010	0.020	0.021	0.028	0.021	0.013	0.031	0.011	0.007
Ho Avg.	0.847	0.803	0.788	0.725	0.811	0.857	0.828	0.809	0.804	0.859	0.822	0.813	0.833	0.782	0.828	0.796	0.863	0.864
Ho Var.	0.005	0.016	0.028	0.041	0.051	0.012	0.027	0.027	0.026	0.013	0.025	0.025	0.027	0.024	0.027	0.024	0.009	0.007
AR 26d Avg.	13.84	11.14	10.15	7.39	11.51	11.62	13.04	11.97	12.04	11.63	11.92	13.18	13.16	12.61	12.13	13.32	13.09	13.82
AR Var.	32.52	23.15	15.46	12.24	21.55	19.89	31.51	26.76	25.44	23.58	26.52	31.71	33.25	30.11	26.56	31.73	33.71	25.07
Obs#A Avg.	17.18	14.71	12.29	7.53	13.35	13.59	16.53	14.00	14.88	13.76	14.65	16.76	15.82	14.41	13.41	13.82	15.41	16.71
Obs#A Var.	54.26	43.80	26.20	10.78	30.86	31.32	53.27	41.05	47.30	27.18	56.56	55.72	54.56	41.72	31.53	29.72	41.06	43.18
F_{IS} (#pos)	13	9	10	2	6	5	9	9	13	5	6	9	7	13	10	13	7	12
F_{IS} (#neg)	4	8	7	15	11	12	8	8	4	12	11	8	10	4	7	4	10	5
F_{IS} (#< -0.05)	1	3	2	11	6	4	2	2	1	3	5	2	2	1	3	2	3	1
F_{IS} (#< -0.075)	1	1	1	8	4	2	0	1	1	1	2	1	1	0	1	2	1	0

Notes:

1. GD Avg. = Average Gene Diversity; GD Var. = Gene Diversity variance; Ho Avg. = Average Observed Heterozygosity; Ho Var. = Observed Heterozygosity variance; AR 26d Avg. = Average Allele Richness standardized to 26 diploid individuals; AR Var. = Variance in Allele Richness; Obs#A Avg. = Average observed number of alleles; Obs#A Var. = Observed number of alleles variance; F_{IS} (#pos) = Number of positive F_{IS} values; F_{IS} (#neg) = Number of negative F_{IS} values; F_{IS} (#<-0.05) = Number of negative F_{IS} values <-0.05; F_{IS} (#<-0.075) = Number of negative F_{IS} values <-0.075.
2. Full sample names corresponding to five-character sample codes are given in Table 1.

Table 3. Pairwise F_{ST} estimates between sample collections obtained from the Southern Upland in years 1999-2009.

	SAD 00	TSK 99	MED 01	LAH 00	GLD 01	MSQ 00	MOS 00	SMA 00	smE 07	smW 07	COU 00	SAG 09
ROH00	0.168	0.131	0.135	0.135	0.138	0.163	0.130	0.139	0.135	0.143	0.134	0.138
SAD00		0.035	0.024	0.054	0.038	0.047	0.063	0.039	0.041	0.050	0.061	0.039
TSK99			0.024	0.036	0.036	0.054	0.049	0.044	0.032	0.035	0.043	0.030
MED01				0.029	0.023	0.043	0.045	0.032	0.022	0.028	0.039	0.024
LAH00					0.030	0.051	0.054	0.050	0.040	0.039	0.040	0.032
GLD01						0.052	0.050	0.037	0.025	0.035	0.036	0.027
MSQ00							0.058	0.027	0.047	0.047	0.050	0.049
MOS00								0.045	0.037	0.047	0.044	0.040
SMA00									0.022	0.033	0.045	0.031
smE07										0.008	0.029	0.014
smW07											0.042	0.025
COU00												0.029

Note: All pairwise estimates significant at $p<0.05$; full sample names corresponding to five-character sample codes are given in Table 1.

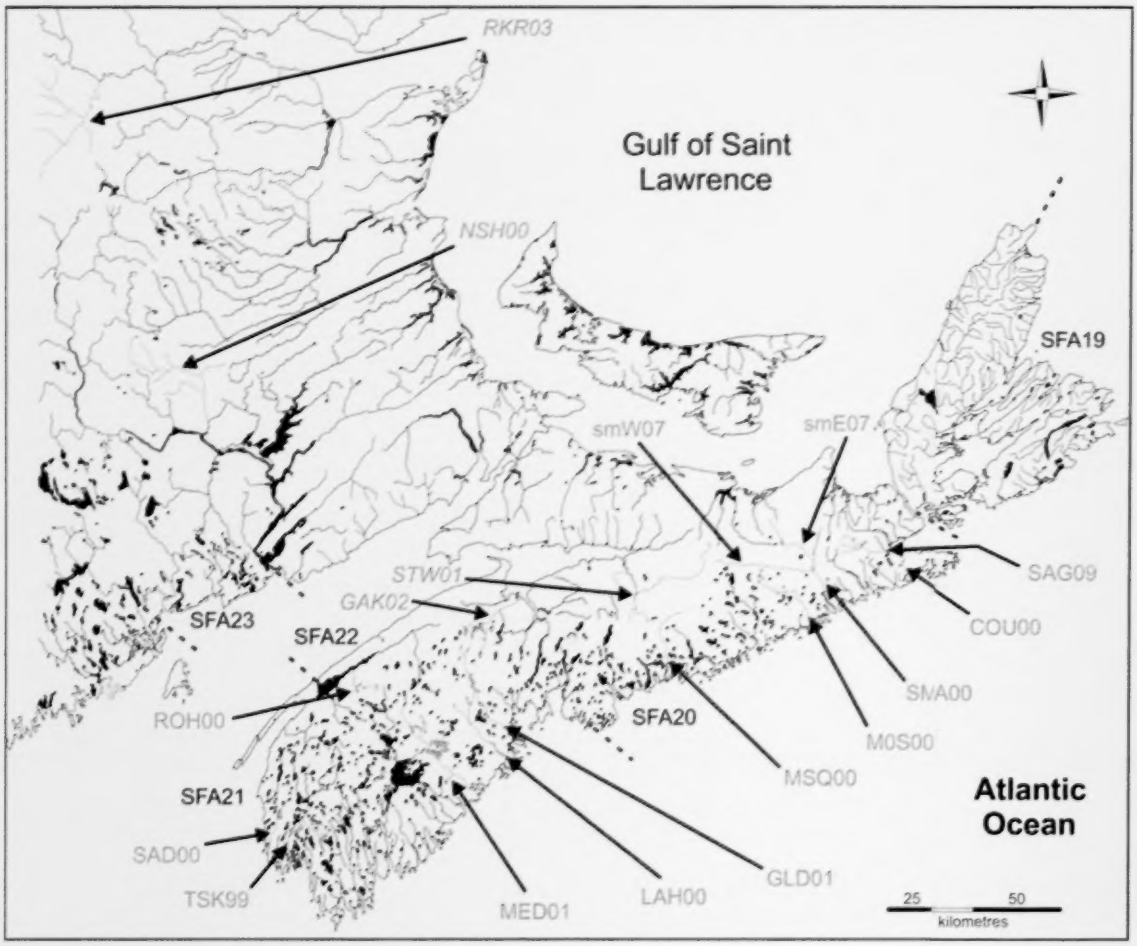

Figure 1. Geographic location of sampled rivers. Southern Upland Rivers are in regular blue font, non-Southern Upland reference rivers are in italicized blue font. Salmon Fishing Areas (SFAs) are given in regular black font. Full sample names corresponding to five-character sample codes are given in Table 1.

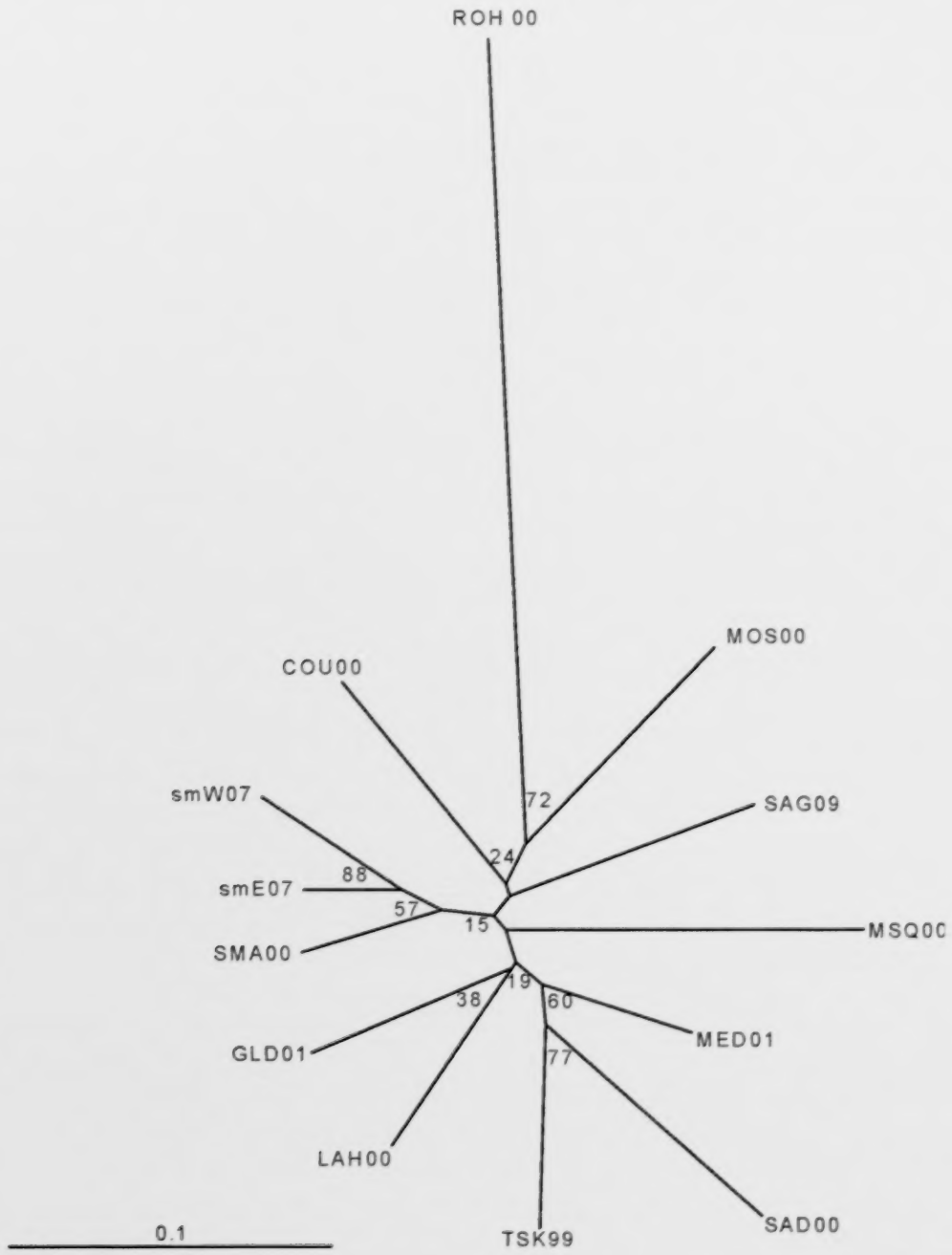

Figure 2. Population phylogram based on Nei's D_A pairwise genetic distances, constructed using the neighbour-joining method. Numbers near branch nodes indicate level of bootstrap support obtained by resampling across loci (with replacement) 1,000 times. Full sample names corresponding to five-character sample codes are given in Table 1.

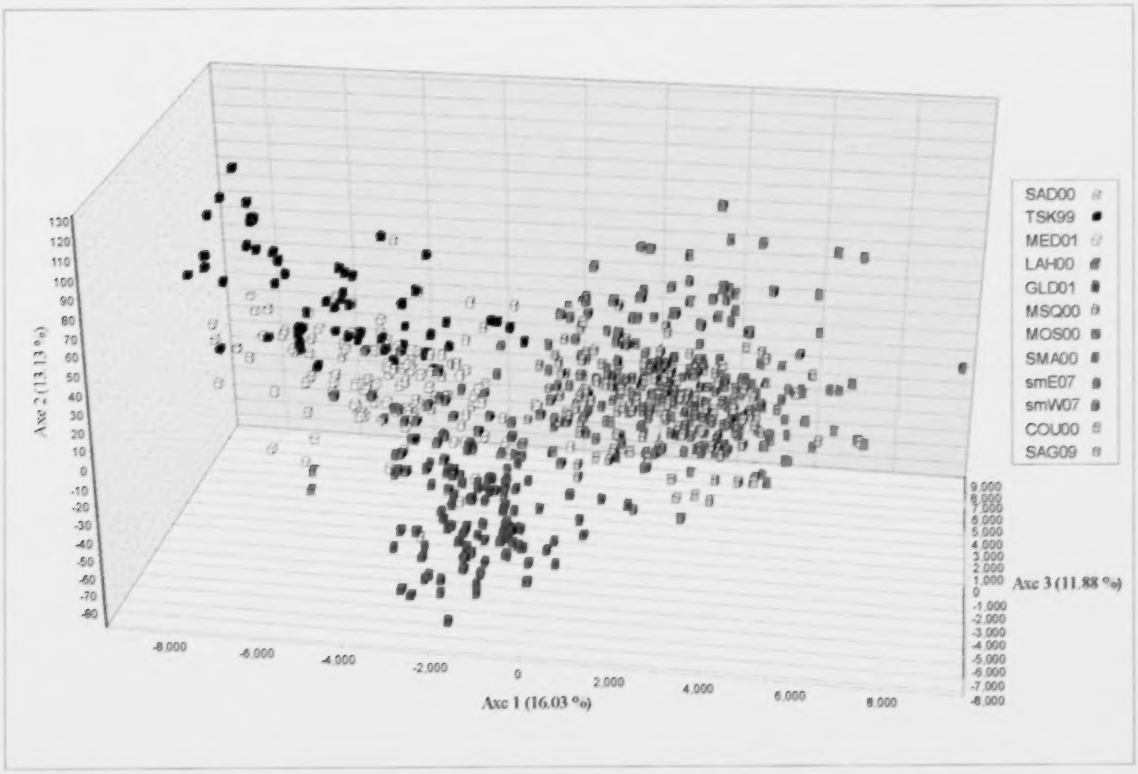

Figure 3. Factorial Correspondence Analysis of multilocus microsatellite genotype information for individuals obtained from all Southern Upland populations surveyed, except Round Hill. Population of origin is identified by colour (see key, top right). Full sample names corresponding to five-character sample codes are given in Table 1.